

## Some aquatic species of Phycomycetes found in Kyoto.

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### Introduction

Recently the writer reported eight species of fungi belonging to Saprolegniaceae (6) and seven species of *Pythium* (7) which had been isolated from water in Kyoto. During the course of the study he succeeded also in making pure cultures of three other *Pythiums* which he considered to be new species. Although he (8) has already described them in Japanese as a preliminary report, their Latin diagnoses have not yet been given.

In the present paper the names of these eighteen species of the aquatic fungi are recorded and in case of need the Latin descriptions as well as the brief notes are given.

### Isolations of the fungi

All the fungi were isolated from water, in which boiled grains of rice or sterilized bodies of house-flies had previously been taken for the sake of obtaining the growing hyphae of the aquatic fungi. Single-cell-cultures were made from sporangia, gemmae or conidia, when they were formed in pure cultures. In order to avoid bacterial contaminations, the writer used RAPER'S method (10). In the present investigation the writer used a number of culture media and found that rice grains in sterilized water are suitable for most of these fungi studied. Corn meal agar (50 g. corn meal, 1000 cc. water and 20 g. agar), oatmeal agar (50 g. oatmeal, 1000 cc. water and 20 g. agar) and 0.05% or 0.025% haemoglobin as liquid medium were also good for usage.

#### 1. *Saprolegnia Thureti* DE BARY

Abh. Senck. naturf. Ges. XII, 1881.

*Saprolegnia ferax* (GRUITH.) THURET (non KÜTZING), Ann. sci. nat. Bot. sér. 3, XIV, 1850.

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[Journ. Jap. Bot. XX-1, (Jan. 1944)]

*Achlya prolifera* PRINGSH. (non NEES), Nova Acta Acad. Leop. XXIII, 1851.  
*Saprolegnia dioica* PRINGSH. (non SCHRÖTER vel DE BARY), Jahrb. wiss. Bot. 11, 1860.

*Saprolegnia dioica* PRINGSH. var. *racemosa* DE LA RUE, Bull. Soc. Imp. Nat. Moscow, XLII, 1, 1869.

*Saprolegnia bodanica* MAURIZIO, Jahrb. wiss. Bot. XXIX, 1896.

Hab. In the fresh water of a pond, Kyoto (2/V, 1934; 12/XII, 1934; 21/XII, 1935).

2. *Saprolegnia mixta* DE BARY

Bot. Zeit. XXXXI, 1883.

Hab. In the fresh water of a pond, Kyoto (12/XII, 1934).

Remarks. This fungus has never been reported from Japan. Its general character is as follows:—

Zoosporangia and gemmae similar to those of *Saprolegnia Thureti*. Oogonia globose, terminal or intercalary, with numerous conspicuous pits on the wall, 41.3-78.8 $\mu$  in diameter. Oospores centric, spherical, 1-20 in an oogonium, 16.7-31.7 $\mu$  in diameter. Antheridial branches diclinous or androgynous and also arising from oogonial stalks, 1-4 to an oogonium, occurring on about 50% of the oogonia in ordinal culture media.

3. *Saprolegnia monoica* PRINGSH. var. *glomerata* TIESENHAUSEN

Arch. f. Hydrobiologie u. Planktonkunde, VII, 1912.

*Saprolegnia furcata* APINIS (non MAURIZIO), Acta Horti Bot. Univ. Latviensis, IV, 1929.

*Saprolegnia glomerata* (TIESENH.) A. LUND, Mém. Acad. Royal, sci. et lett. Danemark, Sec. sci., VI, 1934.

Hab. In water of a ditch, Kyoto (25/X, 1934).  
 Remarks. The present species is also new to Japan. Its general character is as follows:—

Zoosporangia and gemmae similar to those of *Saprolegnia Thureti*. Oogonia globose, usually terminal, with conspicuous pits on the wall, 41.3-78.8 $\mu$  in diameter. Oospores centric, spherical, 2-15 in an oogonium, 13.3-28.7 $\mu$  in diameter. Antheridial branches short, often branched and contorted, usually androgynous and arising from oogonial stalks, rarely diclinous, 1-7 on each oogonium.

4. *Achlya racemosa* HILDEBRAND

Jahrb. wiss. Bot., VI, 1867.

*Achlya lignicola* HILDERBRAND, Jahrb. wiss. Bot., VI, 1867.*Achlya prolifera* K. TAKAHASHI (non DE BARY), Jour. Plant prot., V, 1918  
(in Japanese).

Hab. In the fresh water of a pond, Kyoto (20/XI, 1934).

5. *Achlya imperfecta* COKER.

The Saprolegniaceae with notes on other water molds, 1923.

Hab. In the fresh water of a pond, Kyoto (4/IX, 1934; 19/IX, 1934).

Remarks. The present fungus has never been recorded in Japan. Its general character is as follows:—

Sporangia 100–780×10–45 $\mu$  in size; zoospores 9–13.3 $\mu$  in diameter; gemmae present. Oogonia terminal, globose, usually with conspicuous pits on the wall, 26–80 $\mu$  in diameter. Oospores spherical, eccentric, 13.3–26.5 $\mu$  in diameter, 1–13 in an oogonium. Antheridial branches dielinous or androgynous and also arising from oogonial stalks.

The great majority of the oospores fail to reach at full maturity.

6. *Achlya Oryzae* ITO et NAGAI

Jour. Fac. Agr. Hokkaido Imp. Univ., XXXII, 1931.

Hab. In irrigating water from Lake Biwa, Kyoto (15/VIII, 1934); in water of a ditch, Kyoto (22/VIII, 1934).

Remarks. In 1935 FORBES (5) described three forms of *Achlya* which he considered to exist between *Achlya americana* HUMPHR. and *A. deBaryana* HUMPHR. One of them is almost similar to the fungus in question. Therefore, *A. Oryzae* may be an intermediate form between these two species.7. *Dictyuchus* sp.*Dictyuchus sterile* COKER, The Saprolegniaceae with notes on other water molds, 1923.

Hab. In the fresh water of a pond, Kyoto (12/XII, 1934).

Remarks. The fungus has never produced sexual organs and morphologically agrees with *Dictyuchus sterile* COKER. Judging from the fact that the heterothallism in *Dictyuchus* was reported by COUCH (1) in 1926, *D. sterile* is considered to be a strain of these heterothallic species of *Dictyuchus*.

Therefore, the writer treats the fungus as *Dictyuchus* sp. in this paper.

8. *Aphanomyces helicoides* v. MINDEN

Kryptogamen Flora Brandenburg, V, 1912.

Hab. In the fresh water of a pond, Kyoto (13/XI, 1934).

Remarks. The present species is also a new addition to the mycological flora of Japan. The results of the writer's study are as follows:—

Zoosporangia indistinguishable from hyphae in width; zoospores 8.3–10 $\mu$  in diameter, encysting in a clump at the sporangial tip. Oogonia globose, terminal, 23.3–40 $\mu$  in diameter. Oospores centric or subcentric, spherical, single to an oogonium, 15.8–26.7 $\mu$  in diameter. Antheridial branches dichinous or androgynous, usually winding themselves about each other, and also about other thread, often making a thick tangle in the neighborhood of the oogonia.

9. *Pythium tenue* GOBI

Scriptis Botanicis Holti Univ. Imp. Petropolitanae, XV, 1899–1900.

*Pythium entophytum* SCHENK (non PRINGSHEIM), Verh. d. Pnys-Med. Gesells. in Würzburg, IX, 1859.

Hab. In the fresh water of a pond, Kyoto (13/XI, 1934); in water of a ditch, Kyoto (12/XII, 1934).

Remarks. MATTHEWS (9) reported that she found a variant in a collection containing *Pythium tenue* GOBI, which has antheridia cut off by a septum and larger oogonia than those of GOBI's species. The writer's fungus almost agrees with MATTHEW's variant. However, he found that the formation of the septum cutting off the antheridium is influenced by temperatures. In the writer's fungus, it was able to distinguish a septum cutting off the antheridium when cultured at 24°C., but not at 10–18°C.

According to MATTHEWS, all attempts to grow *P. tenue* as a saprophyte in pure cultures were unsuccessful. But the writer succeeded to isolate the present fungus in the artificial media and attained pure cultures so easily, that the fungus is considered as a saprophytic strain of *P. tenue*. The morphology of the fungus in question is as follows:—

Sporangia filamentous, same size as vegetative hyphae. Oogonia spherical, terminal or interealary, 11.6–19.2 $\mu$  in diameter. Oospores spherical, single, aplerotetic, 9.2–14.9 $\mu$  in diameter. Antheridial branch arising from oogonial stalk, one or two to an oogonium. Antheridia cut off or not cut off by septa.

10. *Pythium gracile* SCHENK

Verhandl. d. Pnys.-Med. Gesells. in Würzburg, IX, 1859.

*Pythium reptans* DEBARY, Pringsh. Jahrb. f. wiss. Bot., 11, 1860.

*Pythium* sp. MARSHALL WARD, Quart. Jour. Micr. sci. n. s. XXIII, 1883.

*Nematosporangium gracile* (SCHENK) SCHRÖTER, Engler-Plantl, Natürl. Pflanzen-Familien, 1, Abt. 1, 1897.

Hab. In the fresh water of a pond, Kyoto (20/XI, 1934; 12/XII, 1934; 20/1, 1935).

Remarks. A number of oogonia were formed on sterilized rice grains in water and also on corn meal agar. Although asexual organs have never been observed by the writer, the present fungus agrees with *Pythium gracile* in the appearance of the sexual organs. The morphological characters of the fungus are as follows:—

Hyphae hyaline, 1-5.8 $\mu$  in width; Oogonia globose, terminal or intercalary, 16.7-25 $\mu$  in diameter; Oospores spherical, aplerotie, single, 13.3-20 $\mu$  in diameter, each containing a large oil drop at maturity; Antheridia diclinous and usually one to an oogonium.

11. *Pythium angustatum* SPARROW

Ann. Bot. XXXXV, 1931.

Hab. In water of a ditch, Kyoto (12/XI, 1934); in the fresh water of a pond, Kyoto (21/XI, 1935).

12. *Pythium torulosum* COKER et PATTERSON

Jour. Eli. Mitch. sci. Soc., XXXXII, 1927.

Hab. In the fresh water of a pond, Kyoto (30/III, 1935).

Remarks. The fungus has never been reported in Japan. Its general characters are as follows:—

Hyphae 2-5 $\mu$  in width; Sporangia producing from large inflated bud-like outgrowths on the mycelium; Oogonia globose, terminal or intercalary, 13.3-20 $\mu$  in diameter; Oospores spherical, plerotie, single in an oogonium, each containing a large oil drop at maturity; Antheridia single to an oogonium, clavate, usually arising from the oogonial stalk or occasionally from hypha bearing the oogonium.

13. *Pythium aphanidermatum* (EDSON) FITZPATRICK

Mycologia, XV, 1923.

*Rheosporangium aphanidermatum* EDSON, Jour. Agr. Res., IV, 1915.*Pythium Butleri* SUBRAMANIAM, Mem. Dept. Agr. India, X, 1919.*Nmatosporangium aphanidermatum* (EDSON) FITZPATRICK, Mycologia, XV, 1923.*Nematosporangium Butleri* (SUBRAM.) SIDERIS, Science n. s. LXXI, 1930.

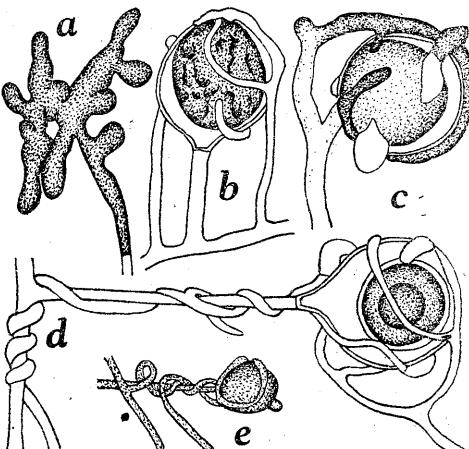
Hab. In irrigating water from Lake Biwa, Kyoto (7/VII, 1934).

14. *Pythium helicum* sp. nov.

Mycelio intra- et extra-matricali; hyphis hyalinis,  $3.3\text{--}6.7\mu$  latis, frequenter multos ramos gemmiformis, zoosporis germinantibus; vesiculis globosis, paucas ad 15 zoosporas gignentibus; zoosporis reniform-biciliatis,  $10\text{--}11.3\times7.5\mu$  circiter; oogoniis terminalibus, sphaeroideis,  $21.6\text{--}35\mu$  diam.; antheridiis androgynis et e pedicello oogonii efformatis vel dielinis 1-6 quoque oogonio adnexit, clavatis, ab caudicula per septum sectis; ramis antheridialiibus longis, oogonium et raro pedicellum oogonii amplectentibus; oosporis solitariis, sphaericis,  $18.3\text{--}28.3\mu$  diam., membrana  $1.2\text{--}3.3\mu$  crassa, in medio globulom grandem continentibus.

Hab. In aqua stagni, Kyoto (15/VIII, 1935; 21/XI, 1935).

Remarks. The bud-like outgrowths of *Pythium helicum* are produced rather in a small number in water cultures and in haemoglobin liquid media, and the formation of laterally biciliate zoospores is observed only in a few occasion. Sexual reproduction takes place abun-

Fig. 1 *Pythium helicum* Ito

- a. Bud-like outgrowth  $\times 238$
- b, c. Young oogonium with androgynous antheridia  $\times 508$
- d. Matured oogonium with dielinous antheridia  $\times 508$
- e. Young oogonium with dielinous antheridia  $\times 238$

dantly in corn meal agar cultures, but the oogonia with accompanying antheridia frequently fail to produce matured oospores and they put forth a germ tube by discharging their degenerated contents.

When the fungus is grown in artificial media, the antheridial branches are wrapping intimately about the oogonium in a manner prevalent among various species of *Aphanomyces*. In other species of this genus as *Pythium graminicolum*, *P. scleroteichum* and *P. perillum*, SUBRAMANIAM (11) and DRECHSLER (2, 4) have already described similar improvement in production of the male organs. *P. graminicolum* and *P. perillum* differ from the writer's fungus especially by the fact that the oospores completely fill the oogonial chamber. In *P. scleroteichum* causing mottle necrosis of sweet potatoes, the bud-like outgrowth as well as the differentiation of zoospores have not been described. The oospores of the latter, which measure 11 to 26 $\mu$  in diameter, are not only smaller than those of the fungus in question, but also differ from it in their distinct yellowish coloration at maturity.

As the writer has not been able to find any species of *Pythium* which agrees exactly with the present fungus, he considers it as a new species.

#### 15. *Pythium proliferum* DE BARY

Jahrb. f. wiss. Bot. II, 1860.

Hab. In a fresh water in the Takano river, Kyoto (27/VII, 1934).

#### 16. *Pythium marsipium* DRECHSLER

Phytopatn. XXXI, 1941.

Hab. In water in a nursery bed of rice plants, Kyoto (22/V, 1935); in the fresh water of a pond, Kyoto (15/VIII, 1935).

Remarks. This species has never reported from Japan. Its general character is as follows:—

Hyphae hyaline, 3.3-5 $\mu$  in width. Zoosporangia globose to pyriform, terminal or intercalary, proliferate, differentiating laterally biciliated zoospores by vesicle, 30-53×24-41 $\mu$  in size. Exit tubes usually very short, often formed asymmetrically to hypha bearing the sporangium. Conidia same size as sporangia, germinating by one or two germ tubes, terminal or intercalary, sometimes catenulated. Oogonia terminal or intercalary, globose, 23.3-35 $\mu$  in diameter. Oospores spherical, single, aplerotie, 18.3-28.3 $\mu$  in diameter, each

containing a large oil drop when mature. Antheridia clavate, 1-12 (usually 1-4) to an oogonium, diclinous.

17. *Pythium polypapillatum* sp. nov.

Mycelio intra- et extra-matricali; hyphis hyalinis, 2.8-8.5 $\mu$  latis; sporangiis terminalibus, globosis, ovoideis vel pyriformibus, 25-35×23.3-30 $\mu$ , proliferis; tubulis exitus apice vel lateraliter instructis, brevibus; vesiculis globosis, 6-15 zoosporas gignentibus; zoosporis reniformibus, lateraliter biciliatis, 13-16.2×11.3-13 $\mu$ ; oogoniis terminalibus, sphaeroideis, membrana echinulata, sine spiculis 16.6-28.3 $\mu$  diam., spiculis tuberiformis, 5-26.3 $\mu$  longis, in basi 2-5 $\mu$  latis; antheridiis androgynis, diclinis, vel raro hypogenis, 1-4 quoque oogonio adnexit, elavatis vel oblongatis; oosporis solitariis, globosis, aplerotieis, 11.6-21.7 $\mu$  diam., in medio globulom granadem continentibus.

Hab. In aqua stagni, Kyoto (21/XI, 1935).

Remarks. The sexual reproduction of *Pythium polypapillatum* takes place abundantly in many nutrient agar media, but sexual organs are produced only in SACHS' nutrient agar media.

*Pythium megalacanthum* DE BARY and *Pythium anandrum* DRECHSLER have been described as the species of *Pythium* characterized by proliferous sporangia and spiculate oogonia. The fungus under consideration is distinguished from *P. megaracanthum* by its smaller zoospores and oogonia as well as the origin of its antheridia, many of which are androgynous. It differs also from *P. anandrum* by the presence of antheridia. According to DRECHSLER

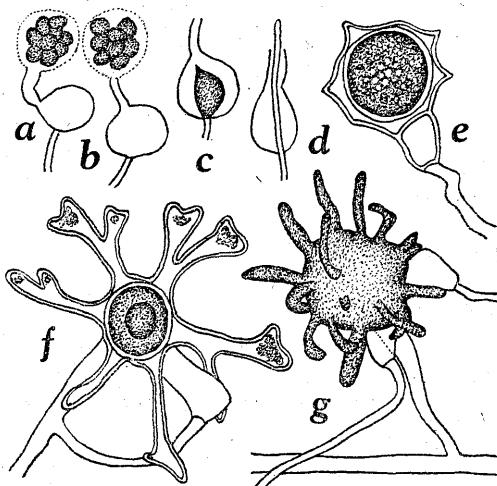


Fig. 2 *Pythium polypapillatum* Ito  
 a, b. Sporangia and vesicles  $\times 235$   
 c, d. Proliferate sporangia  $\times 235$   
 e. Oogonium with a hypogynous antheridium  $\times 524$   
 f. Matured oogonium with an antheridium arising from the oogonial stalk  $\times 524$   
 g. Young oogonium with diclinous antheridia  $\times 524$ .

(3) *P. anandrum* lacks normal antheridia and he observed only the hypogynous antheridium-like bodies.

18. *Pythium pleroticum*, sp. nov.

Mycelio intra- et extra-matricali; hyphis hyalinis, 3-5 $\mu$  latis; conidiis terminalibus vel intercalaribus, globosis, ellipsoideis vel pyriformibus, 18-41 $\mu$  diam., hypha germinantibus; oogoniis terminalibus vel intercalaribus, globosis, 15-18.3 $\mu$  diam.; antheridiis clavatis, 1-4 quoque oogonio adnexit, androgynis et e pedicello oogonii efformatis vel dielinis; oosporis solitariis, sphaericis, pleroticis, membrana 0.5-0.8 $\mu$  crassa, in medio globulom grandem continentibus.

Hab. In aqua stagni, Kyoto (30/III, 1935; 23/-XII, 1940).

Remarks. The conidia of the fungus germinate by producing usually one or two germ tubes, but never differentiate zoospores. The oogonia are plerotic and fertilized by one to four antheridia coming from the oogonial stalk or neighboring hyphae. The oogonial walls and the antheridia are obscure at maturity to an extent that it is impossible to observe exactly.

*Pythium conidiophorum* JOJKL is almost similar to the writer's fungus, but differs in various details. Among those differences the absence of antheridium is recognized to be most important. *Pythium cucurbitacearum* TAKIMOTO, described as a parasite on cucumber, produces many irregularly enlarged

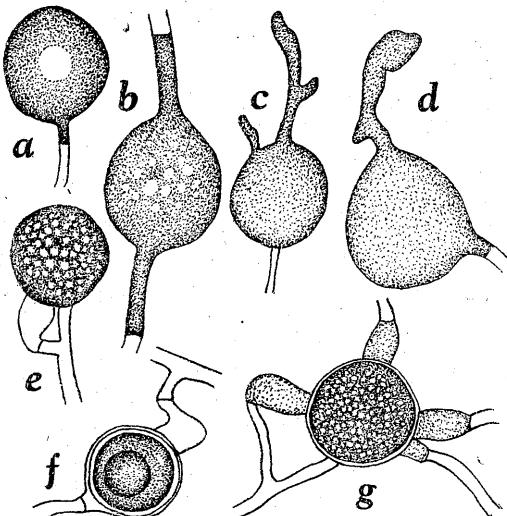


Fig. 3 *Pythium pleroticum* Ito  
 a. Terminal conidium  $\times 754$   
 b. Intercalary conidium  $\times 754$   
 c, d. Germinating conidium  $\times 754$   
 e. Young oogonium with an antheridium arising from the oogonial stalk  $\times 754$   
 f. Matured oogonium with a dielinous antheridium  $\times 754$   
 g. Young oogonium with four antheridia  $\times 754$

hyphal outgrowths in artificial media and moreover its conidia are smaller than those of the writer's fungus. Accordingly the fungus under consideration is recognized to be a new species.

In conclusion the writer wishes to express here his heartiest thanks to Prof. T. HEMMI, under whose direction this study was undertaken, for his many helpfull suggestions. The writer wishes also to acknowledge his indebtedness to Dr. T. ABE and Mr. S. AKAI for adviee and suggestions.

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